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Research paper

Physico-mechanical properties of wound dressing material and its biomedical application

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ABSTRACT

A bioadhesive wound dressing material, based on gelatin, was prepared by solution casting, and its properties were evaluated. The tensile strength (TS) and percentage elongation at break (Eb) of the membranes were found to be 12.7 MPa and 40.4%, respectively. The buffer uptake and water uptake of the prepared membranes were found to be 298 and 312%, respectively, after 8 min. A scanning electron micrograph of the membrane revealed its uniform porosity, which is an essential criterion to be an ideal wound dressing. From microbial sensitivity analysis, it was found that the membrane had a significant resistance against infection. The wound-healing characteristics of the membrane were evaluated using a rat (*Rattus norvegicus*) model. Full-thickness wounds were created on the ventral side of the *Rattus norvegicus* and were dressed with the membrane; eco-plast was used as a control. The wound healing and bioadhesion were monitored at 3-day intervals by real-time imaging. The results revealed that the prepared membrane was more effective in healing the wound than conventional wound dressing.

1. Introduction

Wound healing is a dynamic process, and the performance requirements of the dressing can change as healing progresses. However, it is widely accepted that a warm, moist environment encourages rapid healing, and most modern wound care products are designed to provide these conditions (Winter, 1962; Barnett and Irving, 1991). Wound care often is labor intensive, requiring frequent attention by skilled professionals. Severe wounds (injury or burning) take millions of lives each year all around the world. Severe wounds damage the epithelium or even the endothelium of skin, which is the primary defense barrier of the body (Loke et al., 2000). People die due to severe infection and most likely due to dehydration (Hinrichs et al., 1992; Khil et al., 2003). Conventional wound dressing materials do not provide notable infection resistance. They also lack any water-retaining property to minimize the dehydration. But an ideal wound dressing material should control the wetness and humidity, provide bacterial resistance, and enhance the activities of the growth factors. It should have permeability for oxygen and carbon dioxide, and be able to absorb the wound exudate, and enhance the healing.

Biomaterials have taken part in the development of novel treatments over the last 30 years. The incorporation of natural materials such as gelatin, pectin, starch, cellulose, alginate, chitin, collagen, polyamino acids, hyaluronates, and dextran into synthetic wound dressings has been shown

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to enhance the healing process (Cardona et al., 1996; Grzybowski et al., 1997; Suzuki et al., 1998). The structures of these materials, primarily composed of sugar and/or amino acid residues, are analogs of protein and growth factor structures in the human body that may be more relevant for stimulating the appropriate physiological responses required for cellular regeneration and tissue restructuring in wounds. The development of biopolymers modified with stimulus molecules has added new dimensions in wound dressing for its fulfillment of the requirements to be a perfect wound dresser. An improved dressing can enhance both the rapidity of healing and the quality of the outcome, including reducing infection, pain, and scarring. An improved dressing also can reduce costs, by improving the rate of wound healing and thereby reducing the duration of treatment, and by allowing for less frequent and simpler attention by medical professionals. Improved methods for monitoring wound healing can facilitate better choice of treatment, and reduce costs by allowing for less frequent attention by medical professionals.

Gelatin is a well-characterized protein fragment obtained by partial degradation of water-insoluble collagen fiber, and it has been widely used in the biomedical field, because of its merits, including its biological origin, biodegradability, hydrogel properties, and commercial availability at a relatively low cost. It is also a biocompatible and very low antigenic material. Gelatin has also been used in medicine as a plasma extender wound dressing, an adhesive, and in absorbent pads for surgical use (Choi and Regenstein, 2000). Recently, gelatin has been demonstrated to exhibit activation of microphage (Klose et al., 1952; Wainewright, 1977) and high-hemostatic effects (Montero et al., 1999). Consequently, it has been used in a wide variety of wound dressings and as a biomaterial in tissue engineering (Gennadios et al., 1994).

One of the drawbacks of gelatin for tissue engineering applications is its solubility in aqueous media; therefore, gelatin-containing structures for long-term biomedical applications need to be crosslinked (Venien and Levieux, 2005). In this experiment, polyethylene glycol (PEG) was used as a crosslinker to modify the gelatin membrane as well as to increase the adhesiveness of the membrane. PEGs are water-soluble synthetic polymers, with general formula $HO–(CH_2CH_2O)_n–H$; they are non-toxic, biodegradable, biocompatible, and also non-antigenic (USFDA, 2006). PEGs are widely used in various applications including pharmaceuticals and biotech industries. They are also used as co-solvents, lubricants and stabilizers, bases in topical products, precipitants and crystallization agents for proteins, and as chemical agents for pegylation of proteins. The incorporation of PEG with gelatin has the aim of developing a material which would have good mechanical properties, be thermally stable in the human body, and have good swelling property and effective water absorption capacity; the most important thing is to develop a biocompatible material without any side-effect to the applied natural system. In this research, the design of a wound dressing material based on gelatin was studied. Preliminary laboratory tests as well as preclinical animal study of the produced membranes were conducted for the identification of their usability in wound dressing applications.

2. Materials and methods

2.1. Materials

Type-B gelatin (partial alkaline hydrolysis) was procured from E. Merck, Germany. The molecular weight was 10,000 g/mol. Polyethylene glycol (PEG) was obtained from BDH, England. Injectable ciprofloxacin was purchased from Square Pharmaceutical Ltd., Dhaka, Bangladesh. Rats (*Rattus norvegicus*) were purchased from the Animal Resource Department, ICDDR-B, Mohakhali, Dhaka, Bangladesh.

2.2. Methods

2.2.1. Preparation of gelatin-based membrane

Gelatin granules (10 g) were dissolved in deionized water (100 ml) with continuous stirring at 60 °C to make a viscous solution (the final volume was 50 ml). The solution was autoclaved for 15 min at 121 °C for sterilization. Then the solution was cast at room temperature. The films (membrane) were formed after 48 h of casting. The membranes of gelatin were collected, and then subjected to further drying in vacuum desiccators for 2 days. Then the membranes were stored in desiccators prior to testing. The membranes of gelatin/PEG were also prepared by solution casting. Different percentages of PEG (5–50% w/v) were added to the gelatin solution. The thickness of the membrane was 0.5 ± 0.1 mm.

2.2.2. Antibiotic incorporation and in vitro drug release studies

An antibiotic agent (ciprofloxacin) was incorporated into the gelatin/PEG membrane. Sterile injectable ciprofloxacin was used as the antibiotic agent because of its effectiveness as an antibiotic against air-borne bacteria as well as enterobacteriaceae (Mason et al., 1995; Jeff et al., 2002). Three different doses (0.05%, 0.1%, and 0.2% w/v) of ciprofloxacin-containing membranes were prepared by adding ciprofloxacin solution to the gelatin/PEG solutions during casting of the membrane. A drug release study of the membrane was performed by placing small disks of both antibiotic-added gelatin/PEG membrane and control (gelatin/PEG) membrane on a bacterial lawn. Antibiotic sensitivity (a zone of inhibition) was observed after overnight incubation at 37 °C and compared with that from blank gelatin membrane.

2.2.3. Measurement of pH

The pH of the gelatin/PEG solution was determined using a digital pH meter (Philip, PW-9409, UK) with an efficiency level of ± 0.3.

2.2.4. Mechanical properties

The mechanical properties, namely the tensile strength (TS) and percentage elongation at break (Eb), of the membranes were determined by using a Universal Testing Machine (INSTRON, model 1011, UK) with cross-head speed of 10 mm/min and gauge length of 20 mm. The load capacity was 500 N and the efficiency was within ±1%. The mechanical tests on gelatin-containing PEG membranes were performed at 65% relative humidity and at room temperature to enable identical moisture content.

2.2.5. *Water and buffer uptake*

The experiment was designed to simulate an open exuding wound dressed with gelatin/PEG membranes. Both water and phosphate-buffered saline (PBS) were used to monitor the exudate drainage ability of gelatin/PEG membranes. The PBS was prepared by taking 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 in a 1000 ml volumetric flask; 900 ml deionized water was added. Then the pH was adjusted to pH 7.4 with 0.1 N NaOH and 0.1 N HCl. Finally, additional deionized water was added to make the volume up to the mark. A sponge was cut to fit into a 100 ml glass beaker to approximately 3/4 of its height. The PBS solution was poured into the beaker containing the sponge. The sponge was squeezed and pulled to create a pumping action that accelerated the absorption of PBS. After the sponge was fully soaked, more 0.1 M PBS solution was added to a level of about 1 mm above the sponge's top surface, and the entire beaker was placed in a water bath at 32.5 °C, the equilibrating condition cited from US Pharmacopoeia for transdermal delivery systems (USP, 1995). An approximately 20 × 10 mm weighed gelatin/PEG membrane sample was placed on the top surface of the buffer-soaked sponge. Only one side of the gelatin/PEG membrane was allowed to come into contact with the wet sponge surface. The sample was removed periodically (10, 20, 30, 45, 60, 90, 120, 180, 300 and 480 s), blotted dry with filter paper, and weighed until a constant weight was obtained. The same procedure was used to measure the water uptake; water was used instead of PBS. Both the water uptake and the buffer uptake for the gelatin/PEG membranes evaluated were expressed as

$$\text{Weight gain}(W_g) = (W_t - W_o)/W_o \times 100\%,$$

where

W_t = Weight after uptake

W_o = Initial dry weight of the gelatin/PEG membranes.

2.2.6. *Scanning electron microscopy (SEM)*

Film samples (5 × 5 mm) were deposited on an aluminum holder and sputtered with gold–platinum (coating thickness, 150–180 Å) in a Hummer IV sputter coater. The SEM photographs were taken with a Philips scanning electron microscope (XL 30, Philips, UK) at a magnification of 40,000×, at room temperature. The working distance was maintained between 15.4 and 16.4 mm, and the acceleration voltage used was 5 kV, with the electron beam directed to the surface at a 90° angle and a secondary electron imaging (SEI) detector.

2.2.7. *In vivo wound healing*

The wound-healing characteristics of gelatin/PEG membranes were evaluated using a rat model. All experiments were completed with the approval of the authorities of the Animal Care unit of the Bangladesh Atomic Energy Commission. In this study, a female rat, weighing approximately 213 g, was anesthetized with 5 ml diethyl ether using an inhalation anesthesia. The surgical area was shaved with an electric razor, the rat was strapped to a surgical board, and additional anesthesia was provided via a nose cone. After a deep surgical plane of general anesthesia had been reached, a wound, approximately 1 cm in diameter, was created on the left (lateral) side of the rat using curved blade surgical scissors. Both the epidermal and dermal layers were removed, creating a full-thickness wound with minimal bleeding. Then, four diameters of the wound site were marked and measured using digital calipers and averaged to determine the original wound diameter and area. The wound was then dressed with one of three dressings: (1) eco-plast (control); (2) antibiotic-incorporated gelatin/PEG membranes, and (3) non-antibiotic gelatin/PEG membranes. The gelatin/PEG membranes were cut into sheets and were rehydrated with sterile normal saline immediately prior to use. Each wound was additionally dressed with a 2 cm^2 piece of eco-plast to ensure adherence to the bandage. The surgery was repeated multiple times to give a sample size of 8 rats per treatment per time point.

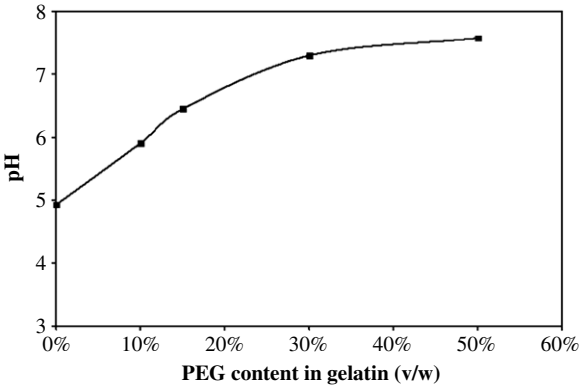

Fig. 1 – Comparison of the pH of gelatin solutions with different PEG content.

3. Results and discussion

3.1. *The pH of the solution*

Gelatin is zwitterionic, and its isoelectric point (IEP) is around 4.8. Thus the pH of its solution is around 5. The pH values of the gelatin solutions were plotted against PEG concentration, and the results are presented in Fig. 1. It was found that the pH of the gelatin solution increased rapidly up to 30% incorporation of PEG then became steady. The rise in pH became a plateau at 50% PEG content. Because of the slightly alkaline nature of PEG (pH 7.6 according to our measurements), it lowers the concentration of the hydronium ion of the gelatin solution and thus improves the pH. It was also noticed that 15% PEG-containing solution had a pH of 6.45, which is close to that of the pH values of skin (~5.5) and blood (7.4 ± 0.04).

3.2. *Morphology and mechanical properties of the membranes*

The morphology of the membranes was investigated by scanning electron microscopy (SEM). The SEM images of (a) a gelatin membrane, (b) PEG; (c) a gelatin/PEG membrane (15% w/v), and (d) gelatin/PEG membrane (30% w/v) are presented in Fig. 2. It was observed that the gelatin (a) had a rough and uneven surface. It is clear that phase separation

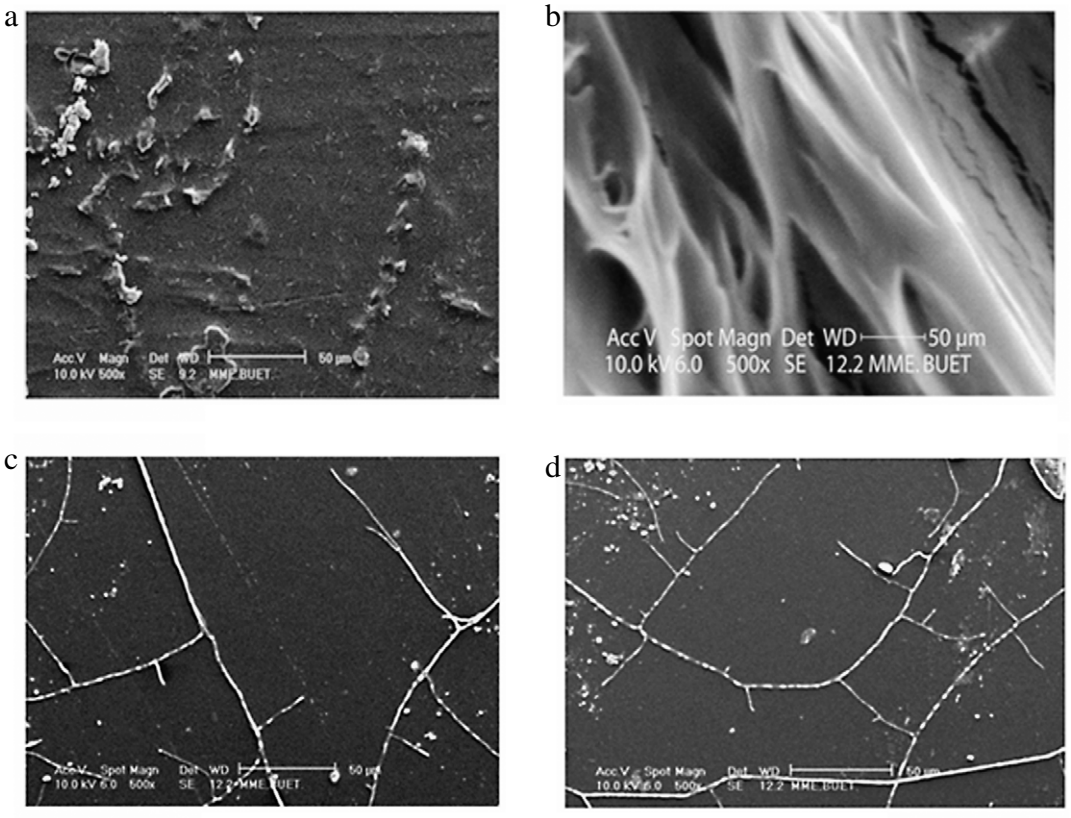

Fig. 2 – Scanning electron micrographs of (a) a gelatin membrane, (b) PEG, (c) a gelatin/PEG membrane (15% w/v), and (d) a gelatin/PEG membrane (30% w/v).

occurred in the gelatin membranes. The micrograph of PEG (b) showed crystalline flat lamellae with leaf-like shape. This feature indicated the morphology of the PEG membrane. Large spaces between the leaf-like structure are available throughout the surface membrane. The crystalline structure was found to form due to the longer evaporation time, which enabled it to crystallize. The SEM images of gelatin/PEG membranes (c–d) indicated network-type morphology. With the increase of PEG, the network structure became more prominent. The degree of phase separation of gelatin in the blended membrane has a significant effect on the strength of the membranes. Therefore, it is necessary to decrease the amount of phase separation to avoid excessive loss of strength of blended membranes. PEG and gelatin have different polarity, and on blending them a degree of phase separation always exists in the sample. Tensile testing provides an indication of the strength and elasticity of the membrane, which can be reflected by the tensile strength and percentage elongation at break. The tensile strength (TS) values are plotted against percentage of PEG content in gelatin (v/w) and are shown in Fig. 3. It was found that the values of TS decreased with increasing percentage of PEG content. The amino group of the gelatin polypeptide chains formed *in situ* acts as an electron donor and the hydrogen of PEG as an electron acceptor. This induces a dipole–dipole attraction between the two phases, which is supposed to enhance the molecular interaction. But this interaction has a larger intermolecular gap, which leads to decrease the TS values. The TS of the membrane exhibited a steady fall with the increase of PEG because the molecular chain structure of gelatin is expected to be somewhat degraded with the incorporation of PEG. It is suggested that the hydrogen of PEG forms a hydrogen bond with the amino group and hence creates the key factor in achieving miscibility. The elongation at break (Eb) of the samples is presented in Fig. 4. It was observed that there was a steady rise of elongation, as expected, with increasing percentage of PEG. The incorporation of PEG molecules into the continuous matrix of gelatin disrupts the structural chain regularities of gelatin, which breaks down the molecular packing and provides a greater path length (path around the periphery of the dispersed particles) for dissipation of energy before its ultimate rupture. However, it also induces some fibrillar characteristics into the system, as observed from the SEM images, which increase its elongation before rupture. For an ideal wound dressing material, the modified gelatin membrane must have a high elongation at break, and good TS (Tanveer et al., 2000). From the study, it was found that 15% PEG content in the gelatin membrane showed a high Eb (40.4%) with moderate TS (12.7 MPa).

3.3. *Fluid drainage ability*

Both the water uptake and buffer uptake of the treated and untreated membranes were monitored periodically (0–480 s) to find the profile of the uptakes. The values of water uptake

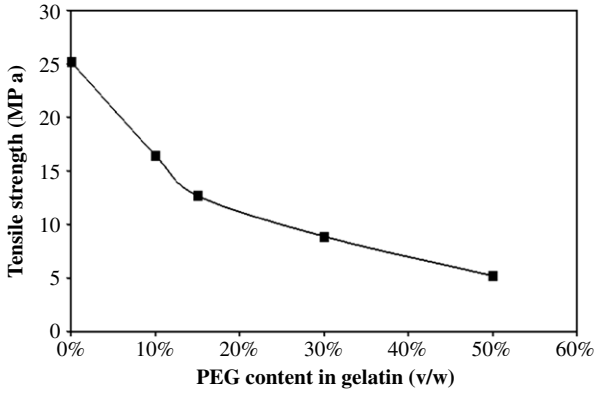

Fig. 3 – Variation of the tensile strength of gelatin membranes with PEG content.

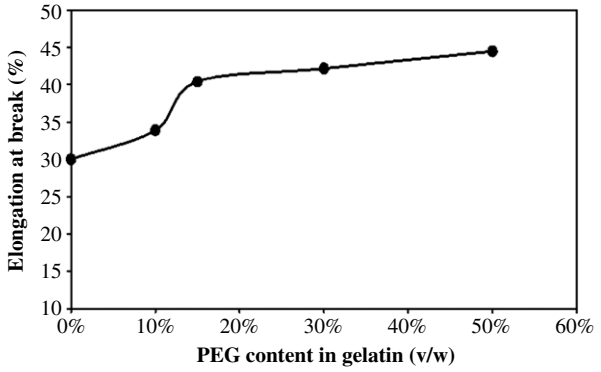

Fig. 4 – Variation of the elongation at break of gelatin membranes with PEG content.

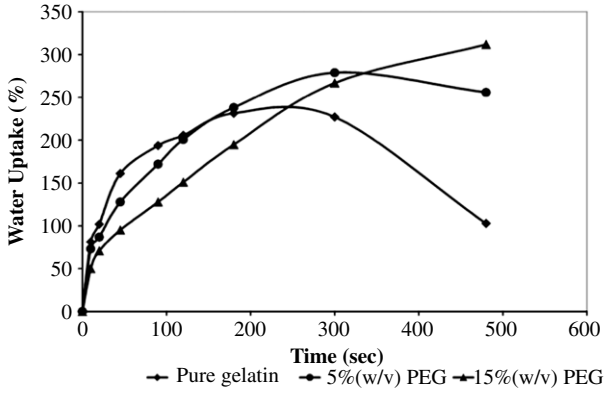

Fig. 5 – Comparison of water uptake of gelatin membranes with different PEG content.

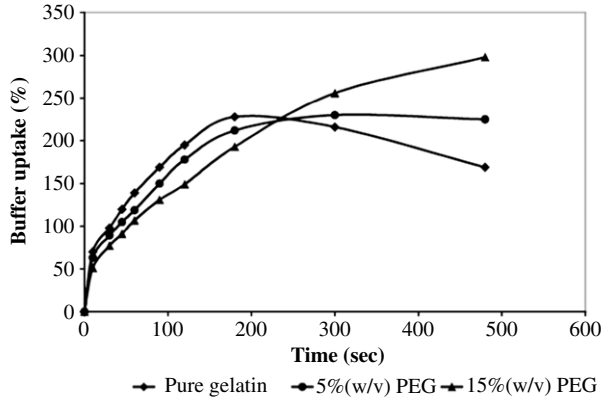

Fig. 6 – Comparison of buffer uptake of gelatin membranes with different PEG content.

and buffer uptake of raw, 5%, and 15% PEG-containing gelatin membranes were plotted against time, and the results are presented in Figs. 5 and 6. It was found that both the water and buffer uptakes increased with time up to a certain level (~200 s). But after that, they began to decrease. Both gelatin and PEG are hydrophilic, and so the water uptake is quite rapid. But after 3 min, the gelatin membrane was saturated, and it began to dissolve. Thus the net weight of the membrane decreased, which decreased the uptake. But, in the case of the PEG-containing membrane, increasing the PEG content increased the water saturation point (above 8 min for 15% PEG content) and thus higher water uptake and buffer uptake were obtained. It was noticed that the water uptake and buffer uptake of the 15% PEG-containing membrane were 312% and 298%, respectively. These values indicated that gelatin is more hydrophilic than PEG and that the interaction of gelatin and PEG caused a lower hydrophilicity. The higher water adsorption ability of the asymmetric gelatin membranes can be attributed to the increased porosity of sponge-like sublayers that resulted in the increase of void to capillary adsorbed water. This result revealed that the higher porous asymmetric gelatin membranes could have the potential to prevent wounds from accumulation of fluid by the adsorption of exudate.

3.4. *In vitro drug release study*

Fig. 7 shows (a) a bacterial (*bacillus spp*) culture, (b) the inhibition zone of a ciprofloxacin disc, (c) the inhibition zone of pure gelatin, and (d) bacterial growth inhibition by an antibiotic-incorporated gelatin/PEG membrane. Drug release at the wound surface was simulated in agar medium. Ciprofloxacin is highly soluble in water (Bayer Health Care Pharmaceutucals, 2009). So when it is poured (either in pure form or incorporated in the membrane) on a nutrient agar plate, it quickly diffuses through the agar medium and thus the concentration of it gradually decreases. So, bacterial growth is found beyond the minimum concentration (MIC), which is sufficient to inhibit bacteria. It was found that the inhibition zone created by ciprofloxacin (0.1% w/v)-added gelatin/PEG membrane is quite similar to the inhibition zone formed by the ciprofloxacin disc Fig. 7(b). This indicates that ciprofloxacin remains quite active when incorporated in a gelatin/PEG membrane. The results also reveal that gelatin itself has some sort of antibacterial action Fig. 7(d).

3.5. *In vivo wound healing*

In the rat model, an artificial wound was formed surgically, and dressed with the developed wound dressing materials

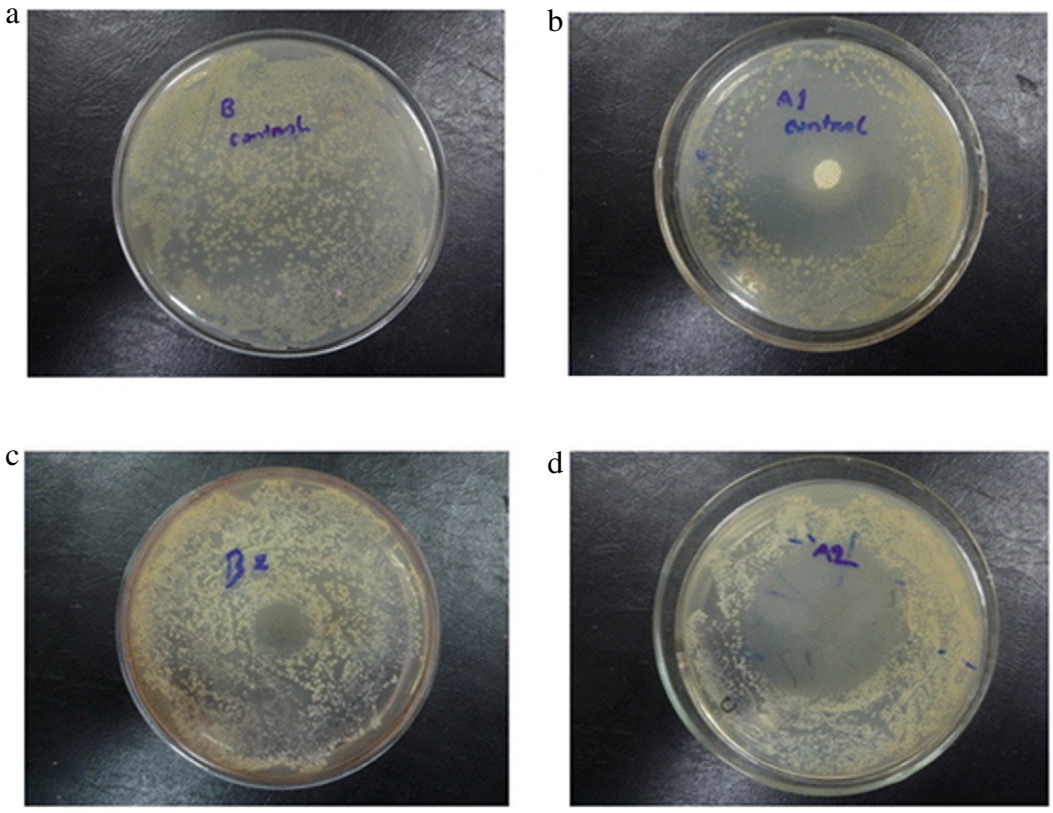

Fig. 7 – (a) Bacterial (*bacillus spp*) culture, (b) the inhibition zone of a ciprofloxacin disc, (c) the inhibition zone of pure gelatin, and (d) bacterial growth inhibition by an antibiotic-incorporated gelatin/PEG membrane.

and also with available wound dresser (eco-plast) as a control. Eco-plast, a thin adhesive membrane coated with a synthetic layer, is permeable to both water vapor and oxygen, but it is impermeable to microorganisms. It is used clinically in the treatment of minor burns, pressure areas, donor sites, and a variety of minor injuries, including abrasions and lacerations, and was selected for these experiments so that effects due to the bioadhesive gelatin/PEG membrane could be assessed in a controlled environment. Wound healing was assessed by monitoring the wound contraction, re-epithelialization, and wound morphology (by real-time photography). Wounds dressed with the new materials showed improved wound-healing results compared to wounds dressed with the eco-plast alone. In addition, the underlying fibro-vascular tissue appeared more rapidly than for wounds treated with eco-plast alone. It was also found that rats that were dressed with gelatin/PEG membranes had a notable healing within six days. But for the eco-plast dressing, fibrin and blood penetrated the large interstices of the fabric, hardened, and stuck firmly to the wound bed, leading to considerable tissue trauma and bleeding during redressing, and consequently delayed healing. During wound healing, no significant weight loss or fever was found. The edges of the wound pulled inwards to reduce the overall wound area. Combining the gelatin/PEG membranes with ciprofloxacin created a wound-healing environment that appeared to meet the criteria set forth above for ideal wound dressing. First, no signs of bacterial infection were apparent during gross examination of the wounds or histologically, suggesting that the materials effectively protected the wound from bacterial infection. Second, the wounds treated with gelatin/PEG membranes were moist and hydrated, thus demonstrating that evaporative water loss and wound dehydration had been prevented. Third, abundant cellular proliferation suggested that oxygen and carbon dioxide permeability had been maintained. Fourth, the absorption of wound exudates was clearly visible in those wounds treated with gelatin/PEG membranes. Finally, the acceleration of re-epithelialization with the gelatin/PEG membrane treatment indicated an enhancement of the overall healing process. The mechanisms by which these gelatin/PEG membranes accelerate re-epithelialization are as yet unknown. However, reasonable explanations can be proposed. It is probable that the gelatin membranes promote cell movement in early granulation tissue. Gelatin has been found to bind and act as a repository for a large family of cytokines (Wood, 1960). It is likely that the gelatin/PEG membranes are behaving as an artificial extra cellular matrix (ECM), retaining cytokines and other growth factors made by the regenerating tissue. Wound exudate mixed with degrading membrane was clearly visible upon gross and histological examinations. Therefore, it is believed that these gelatin/PEG membranes provide a highly hydrated, pericellular environment that simulates the ECM. In this growth-conducive environment, the assembly of other matrix components, presentation of growth and differentiation factors, and cell migration all contribute to accelerated wound repair.

4. Conclusions

In this research, a bioadhesive wound dressing material based on gelatin was prepared, characterized, and evaluated for biomedical application. The mechanical and physical properties reveal that 15% w/v PEG-containing membranes show optimum physical and mechanical properties. The bacterial sensitivity test revealed that the produced membrane has excellent bacterial resistance. The gelatin/PEG membrane dressings were found to accelerate wound healing, which progressed more smoothly to maturation compared with the commercial controls. The wound model study showed that all the wounds dressed with gelatin/PEG eventually healed and closed on a macroscopic level. It was also found that the total healing procedure was very cost effective. There was no need for trained personnel because there was no requirement for redressing, due to the membrane's bio-acceptability. The cumulative cost of therapy and intensive nursing care associated with the use of conventional wound dressings shows the cost-effectiveness of this new product. So, from various aspects of wound healing it can be said that use of this newly prepared membrane could be extended to use for bigger wounds. Thus this research has revealed a successful development and application of a new bioadhesive wound dressing material based on modified gelatin which is more effective that conventional wound dressing materials.

REFERENCES

Barnett, S.E., Irving, S.J., 1991. Studies of wound healing and the effect of dressings. In: Szycher, M. (Ed.), High Performance Biomaterials. Technonic, Lancaster, pp. 583–620.

Bayer Health Care Pharmaceutucals, 12 February 2009. CIPRO (ciprofloxacin hydrochloride) TABLETS CIPRO (ciprofloxacin*) ORAL SUSPENSION (PDF). USA: FDA. http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/019537s073,020780s030lbl.pdf. Retrieved 8 September 2009.

Cardona, L.R., Sanzgiri, Y.D., Benedetti, L.M., Stella, V.J., Topp, E.M., 1996. Application of benzyl hyaluronate membranes as potential wound dressings: evaluation of water vapour and gas permeabilities. Biomaterials 17, 1639–1643.

Choi, S.S, Regenstein, J.M., 2000. Physicochemical and sensory characteristics of fish gelatin. J. Food Sci. 65, 194–199.

Gennadios, A., McHugh, T.H., Weller, C.L, Krochta, J.M., 1994. Edible coatings and films based on proteins. In: Krochta, J.M., Baldwin, E.A., Nisperos-Carriedo, M. (Eds.), Edible Coatings and Films to Improve Food Quality. N.C. Technomic Pub.Co. Inc., Lancaster, pp. 201–278.

Grzybowski, J., Kolodziej, W., Trafny, E., Struzyna, J., 1997. A new anti-infective collagen dressing containing antibiotics. J. Biomed. Mater. Res. 36, 163–166.

Hinrichs, L.J, Lommen, E.J, Wildevuur, C.R.H, Feijen, J., 1992. Fabrication and characterization of an asymmetric polyurethane membrane for use as a wound dressing. J. Appl. Biomater. 3, 287–303.

Jeff, Zahller, Philip, S., Stewart,, 2002. Transmission electron microscopic study of antibiotic action on klebsiella pneumoniae biofilm. Antimicrob. Agents Chemother. 46, 2679–2683.

Khil, Myung-Seob, Cha, Dong-Il, Kim, Hak-Yong, Kim, In-Shik, Bhattarai, Narayan, 2003. Electrospun nanofibrous polyurethane membrane as wound dressing. J. Biomed. Mater. Res. Part B, Appl. Biomater. 67B, 675–679.

Klose, A.A., Machi, E.P., Hanson, H.L., 1952. Use of antioxidants in the frozen storage of turkeys. Food Technol. 6, 308–311.

Loke, Weng-Keong, Lau, Sok-Kiang, Yong, Lim Lee, Khor, Eugene, Sum, Chow Kok, 2000. Wound dressing with sustained antimicrobial capability. J. Biomed. Mater. Res. (Appl. Biomater.) 53, 8–17.

Mason, D.J., Power, E.G., Talsania, H., Phillips, I., Gant, V.A., 1995. Antibacterial action of ciprofloxacin. Antimicrob. Agents Chemother. 39, 2752–2758.

Montero, P., Gòmez-Guillèn, M.C., Borderias, A.J., 1999. Functional characterization of muscle and skin collagenous material from hake (*Merluccius merluccius*L.). Food Chem. 65, 55–59.

Suzuki, Y., Nishimura, Y., Tanihara, M., Suzuki, K., Nakamura, T., Shimizu, Y., Yamawaki, Y., Kakimura, Y., 1998. Evaluation of a novel alginate gel dressing: cytotoxicity to fibroblasts in vitro and foreign-body reaction in pig skin in vivo. J. Biomed. Mater. Res. 39, 317–322.

Tanveer, Ahmad Khan, Kok, Khang Peh, Hung, Seng Ch'ng, 2000. Mechanical, bioadhesive strength and biological evaluations of chitosen membranes for wound dressing. J. Pharm. Sci. 3 (3), 303–311.

USFDA. Interactive Ingredient Guide (Redacted) January 2006. http://www.fda.gov/cder/drug/iig/default.htm (accessed August 26, 2006), part of www.fda.gov/cder (accessed August 26, 2006).

USP, 1995. Drug release. Transdermal delivery systems—General drug release standards. United States Pharmacopeia and National Formulary 724, pp. 1796 –1798.

Venien, A., Levieux, D., 2005. Differentiation of bovine from porcine gelatins using polyclonal anti-peptide antibodies in indirect and competitive indirect ELISA. J. Pharm. Biomed. 39, 418–424.

Wainewright, F.W., 1977. Physical tests for gelatin and gelatin products. In: Ward, A.G., Courts, A. (Eds.), The Science and Technology of Gelatin. Academic Press, New York, pp. 507–534.

Winter, G.D., 1962. Formation of the scab and the rate of epithelialization of superficial wounds in the skin of the young domestic pig. Nature 193, 293–294.

Wood, G.C., 1960. The formation of fibrils forms collagen solutions. Effect of chondroitin sulfate and other naturally occurring polyanions on the rate of formation. Biochem. J. 75, 605–612.